(19) KOREAN INTELLECTUAL PROPERTY OFFICE (KR)
(12) PATENT LAID-OPEN GAZETTE (A)

(51) ○ Int. Cl. [7] (11) Laid-Open Publication No.: 10-2005-004407
A61k 35/78 (43) Laid-Open Publication Date: May 12, 2005
(21) Application No. 10-2003-0078503
(22) Filing Date: November 7, 2003
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Request for Examination: Yes

(54) COMPOSITION FOR PREVENTING AND TREATING BRAIN DISEASE INCLUDING GRAPE SEED EXTRACT HAVING NEURONAL CELL PROTECTING ACTIVITY

ABSTRACT

The present invention relates to a grape seed extract having neuronal cell protecting activity induced by ischemia and a composition comprising the same for preventing and treating degenerative brain disease. The grape seed extract shows potent inhibiting activity of neuronal cell apoptosis occurred in brain ischemia as well as no toxicity, therefore, it can be used as the therapeutics or health food for treating and preventing degenerative brain diseases.

Representative Figure: Fig. 5

Index: ischemia, neuronal cell death, ischemia inhibiting material, grape seed

SPECIFICATION

Brief Description of the Drawings

Fig. 1 shows the preventive effect of grape seed extract administrated into PC12 cell line on the neuronal cell death in the low oxygen environment determined by LDH determination experiment.

Fig. 2 shows stained photographs of the hippocampus region in the brain tissue of experimental animal (A and C are normal groups four days after the ischemia-reperfusion treatment, B and D are control groups treated with only solvent, C and D are magnified photographs of A and B in the region of CA1 (400×)).

Fig. 3 shows stained photographs of the hippocampus region in the brain tissue of experimental animal four days after the ischemia-reperfusion treatment (A and C are photographs of 30 mins before the treatment and B and D are photographs of 30 mins after the treatment).

Fig. 4 represents magnified photograph of CA1 region in Hippocampus tissue depicted in Fig. 3 (400×).

Fig. 5 represents the comparison of the number of viable cells between control group and test groups four days after the treatment with grape seed extract to the experimental animals 30 mins before and after the ischemia-reperfusion treatment.

Detailed Description of the Invention

Objects of the Invention

Background Art

The present invention relates to a grape seed extract having neuronal cell protecting activity and a composition comprising the same for preventing and treating degenerative brain disease. More specifically, the present invention relates to a grape seed extract for preventing or improving damages of hippocampus brain cells caused in PC12 cell line and animal models after ischemia-reperfusion and a composition comprising the same for preventing and treating degenerative brain disease.

According to the ratio of older people reported from the Korean National Statistical Office in October, 2003, the population ratio of 65 or more-year-old people in 2000 reached 7.2% based on the total population so that Korea enters an aging society. The ratio is expected to be more than 14% in 2019. As the aging society problem has recently come to the front as a social issue, people are concerned about welfare including residence, health, culture and leisure of old people and the resulting statistical

demand has been increased. This change results from increase of older people's population. They have died from chronic degenerative diseases rather than acute infectious diseases for the past 50 years. Cerebrovascular disease of the chronic degenerative diseases ranked second in the death rate by single disease. Cerebrovascular disease is classified into two types, hemorrhagic brain disease and ischemic brain disease: hemorrhagic brain disease such as cerebral hemorrhage occurred mainly by some traffic accidents and the ischemic brain disease frequently occurring in older people is caused by the occlusion of cerebral vessels.

In case that temporary cerebral ischemia occurs, the supply of oxygen and glucose to brain is prevented and several syndrome such as ATP decrease and edema follows, which causes to exclusive range of brain injury as the result. After the considerable time lapses, the apoptosis of neuronal cell occurs, which is called as delayed neuronal death. The effect on the delayed type neuronal death is performed by experimenting transient forebrain ischemic model using Mongolian gerbil and it has been reported that neuronal cell death occurs at CA1 region in hippocampus four days after the inducement of cerebral ischemia for 5 mins (kino T et al, *Acta Neuropathol.*, 62 pp201-208, 1984; Krino T, *Brain Res.*, 239, pp57-69, 1982).

It has been reported that the neuronal cell death is caused by two mechanisms; one is excitation neuronal cell death mechanism; cerebral ischemia causes to excessive accumulation of outer glutamate and those glutamate are influxed into inner cell, which causes to neuronal cell death by excessive accumulation of intracellular calcium ion (Kang T. C. et al, *J. Neurocytol.*, 30, pp945-955, 2001) and another is oxidation neuronal cell death; the ischemia-reperfusion causing abrupt supply of oxygen resulting in the increase of internal radical ion to give rise to the injury of DM and cytoplasm (Won M. H. et al, *Brain Res.*, 836, pp70-78,1999 ; Sun A. Y. et al, *J. Biomed.* Sci., 5, pp401-141, 1998; Flowers F et al, *New Horiz.* 6, ppl69-180, 1998).

On the base of those mechanism study, there have been endeavored to develop effectively inhibiting substance of neuronal cell death or the mechanism thereof till now, however, the effective and satisfactory inhibitors of neuronal cell death have been not found yet.

Tissue plasminogen activator, sole approved cerebral ischemia treating agent in FDA and sold in the market is a thrombolytic agent dissolving thrombus causing cerebral ischemia and inducing rapid supply of oxygen and glucose. Accordingly, it

could not protect neuronal cell directly, therefore it should be used urgently. Furthermore, since it is thrombolytic agent, hemorrhagic cerebral disease occurs in case that it is administrated in over dose or too frequently.

MK-801, a potent calcium channel blocker effectively inhibiting initial calcium ion influx had been on clinical trial however it has been abandoned because of its adverse action.

In South Korea, lots of health care food containing natural substance have been on the market however most of those are not yet authorized by scientific test and abused to give rise to scientific problems in the end.

Accordingly, there have been still needed to develop novel natural resource effective in treating and preventing cerebral disease through substantive and scientific experiments till now.

It have been reported that grape seed extract contains high amount of catechin showing various activities such as intoxifying, bacteriocidal and anticancer activities and picnagerol showing strengthening blood vessel and shows antioxidant effect to inhibit the action of harmful oxygen.

The present inventors has found out that that the grape seed extract inhibits the neuronal cell death and show neuronal cell protective activity to complete the present invention.

Technical Subject of the Invention

It is an object of the present invention to provide a natural food extract for preventing neuronal cell damages by ischemia which is harmless to human body.

It is an object of the present invention to provide a grape seed extract having neuronal cell protecting activity.

It is an object of the present invention to provide a composition for preventing and treating brain diseases including the grape seed extract as an active ingredient.

Constitution of the Invention

In order to achieve the above-described objects, the present invention provides a grape seed extract having neuronal cell protecting activity.

Also, the present invention provides a composition for preventing and treating brain diseases including the grape seed extract having neuronal cell protecting activity as an active ingredient.

The grape seed extract is selected from the group consisting of water, lower alcohol of C_1-C_4 and the mixture thereof.

Hereinafter, the present invention is described in detail.

The inventive grape seed extract can be prepared by follows:

(a) At the first step, grape seed is dried, crushed and mixed with 1 to 20-fold, preferably, approximately 8 to 12 fold volume of water to the weight (kg) of the grape seed. After stirred at the temperature ranging from 20 to 50 °C, preferably at room temperature , for about 1 hour to three days, preferably from 12 hours to 1 day, the seed is extracted with strong base like sodium hydroxide and potassium hydroxide adjusted to the pH ranging from 8 to 11, preferably 10, for about 1 to 24 hours.

(b) At the second step, the extract solution is neutralized with acidic solution such as strong acid for example, hydrochloric acid to adjust to the pH ranging from 2 to 4, preferably 3, and then centrifuged to obtain a precipitated layer.

(c) At the third step, about 3 to 7, preferably 5, fold weight of lower alcohol such as methanol or ethanol is added in the precipitant, suspended, centrifuged to obtain a supernatant layer. The supernatant is decompressed to obtain its concentrates.

(d) At the fourth step, an organic solvent such as hexane is added in the concentrate with the same amount, and hexane, which is a supernatant layer, is removed. The residual solvent is purified and lyophilized, thereby obtaining a grape seed extract.

Accordingly, the present invention also provides a grape seed extract having potent neuronal cell protective activity obtained from the above-described preparation method.

In the above preparation method, when the extract solvent has a pH of less than 8.0 after sodium hydroxide is added, the extract efficiency of enzyme inhibiting agents is reduced. As a result, the pH of the extract solvent is adjusted to be more than 8.0.

Grape seed is dried, crushed and mixed with 5 to 20 fold volume of C_1-C_4 lower alcohol to the weight (kg) of the grape seed or water and mixtures thereof, preferably 50 to 100% ethanol. The resulting mixture is precipitated 1 to 5 times at room temperature to 100°C for about 1 to 24 hours, stir-extracted, water-extracted, reflux-cool-extracted, decompressed, concentrated or dried to obtain the grape seed ethanol extract.

Additionally, after the water soluble extract or lower alcohol soluble extract is suspended in water, about 1 to 10 fold, preferably about 1 to 5 fold volume of non-polar solvent such as lower acetate like ethylacetate, chloroform, acetone, dichloromethane, carbon tetrachloride is added in the suspended solvent. The resulting solvent is fractioned one to five times, preferably two to four times to obtain a non-polar solvent soluble layer and a water soluble layer. Also, an additional fraction process may be performed (Harborne J.B.; Phytochemical methods: *A guide to modern techniques of plant analysis*. 3rd Ed., p 6-7, 1998).

In the preferred embodiment of the present invention, the resulting solvent is extracted and concentrated with ethanol. After the same amount of hexane is added and shaken in the concentrated solvent, the hexane layer, which is a supernatant layer, is removed. The residual solvent is lyophilized to obtain a brown powder.

The grape seed extract disclosed herein refers to all the seed extract extracted from Vitis genus such as *Vistis vinifera L, Vitis vinifera, Vitis labrusca, Vitis riparia, Vitis rupestris, Vitis berladieri, Vitis coignetiae Pulliat ex Planchon, Vitis amurensis Ruprecht, Vitisficifolia Bunge, Vitis flexuosa Thunb* and so on.

The present inventors have performed several experiments such as in vitro test determining inhibiting effect on LDH enzyme in PC12 cell line and in vivo animal test using Mongolian gerbil to protect neuronal cells of CA1 region and finally confirmed that the grape seed extract inhibit the neuronal cell death and show potent preventing activity of the neuronal cell injury caused by brain ischemia.

The present invention provides a grape seed extract having neuronal cell protecting activity obtained by the above-described preparation method, which can be used to prevent and treat brain diseases caused by neuronal cell death.

The present invention provides a composition for preventing and treating brain disease including a grape seed extract having neuronal cell protecting activity as an active ingredient.

The brain disease caused by neuronal cell death includes stroke, cerebral concussion, Parkinson's disease, Huntington's disease, Pick disease and Creutzfeld-Jakob disease.

The inventive composition for treating and preventing degenerative brain disease by protecting neuronal cell may comprises above extracts as 0. 001-50 % by weight based on the total weight of the composition. If the amount of seed extract is less than 0.001 % (w/w), over dosing administration may be required to obtain effective efficacy and if the amount of seed extract is more than 50 % (w/w), it is not economical since the efficacy of abundant extract may be equal to that of lesser amount of extract. wever, it is preferable that the amount of extract is controlled according to the using method and the using purpose of the composition.

The inventive composition may additionally comprise conventional carrier, adjuvants or diluents in accordance with a using method well known in the art.

The composition according to the present invention can be provided as a pharmaceutical composition containing pharmaceutically acceptable carriers, adjuvants or diluents, e. g. , lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starches, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, microcrystalline cellulose, polyvinyl pyrrolidone, water, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate and mineral oil.

The composition of the present invention can be used for drugs or food. In case of drugs, it can be administered orally or non-orally. The formation of the composition can be prepared as plasters, granules, lotions, powders, syrups, liquids and solutions, aerosols, ointments, fluidextracts, emulsions, suspensions, infusions, tablets, injections, capsules and pills.

The desirable dose of the inventive extract or composition varies depending on the condition and the weight of the subject, severity, drug form, route and period of administration, and may be chosen by those skilled in the art. However, in order to obtain desirable effects, it is generally recommended to administer at the amount ranging from 50mg/kg to 500mg/kg per day, by weight/day of the inventive extract or compounds of the present invention.

The above described seed extract can be used as main and sub materials of other foods and food additives.

The present invention provides nutraceuticals for preventing and improving brain disease including a grape seed extract and sitologically acceptable food subsidiary addtives.

The composition including the grape seed extract can be used in drugs, food and beverage for preventing and treating brain disease. Examples of addable food comprising above extracts of the present invention are various food, beverage, gum, tea, vitamin complex, health improving food and the like.

The extract of the present invention has neither toxicity nor side effects so that it can be safely used as drug for a long period.

In order to prevent and improve brain disease, the extract of the present invention can be added in food or beverage.

Providing that the health beverage composition of present invention contains above described extract as an essential component in the indicated ratio, there is no particular limitation on the other liquid component, wherein the other component can be various deodorant or natural carbohydrate etc such as conventional beverage. Examples of aforementioned natural carbohydrate are monosaccharide such as glucose, fructose etc; disaccharide such as maltose, sucrose etc; polysaccharide such as dextrin, cyclodextrin; and sugar alcohol such as xylitol, sorbitol and erythritol etc. As the other deodorant than aforementioned ones, natural deodorant such as taumatin, stevia extract such as levaudioside A, glycyrrhizin et al., and synthetic deodorant such as saccharin, aspartam et al., may be useful favorably. The amount of above described natural carbohydrate is generally ranges from about 1 to 20 g, preferably 5 to 12 g in the ratio of 100 ml of present beverage composition.

The composition for preventing and treating brain disease can be used for treating and preventing brain disease caused by neuronal cell death, specifically ischemia.

The present invention is more specifically explained by the following examples. however, it should be understood that the present invention is not limited to these examples in any manner.

Example 1. Preparation of extract of grape seed

Water was added in a grape seed to adjust pH to obtain an extract according to an extract method of the present invention. Also, an extract was obtained by a conventional ethanol extract method.

1-1. Preparation of water extract of grape seed

1 kg of dried and crushed grape seed was mixed with 10 folds volume of distilled water and stirred. Appropriate amount of NaOH was added thereto to adjust the pH to 10 and stirred to extraction for 6 hours at R. T. The obtained extract was acidified with HCl to adjust the pH to 3.0, subjected to centrifugation to collect 100g of precipitates. 5 folds of ethanol (w/w) was added to the concentrates, suspended and subjected to centrifugation to obtain the supernatant. The supernatant was concentrated to obtain 50g of concentrates and equal amount of hexane was added thereto to remove a hexane soluble layer. A remaining lower layer was dried with lyopholization to obtain 30g of brown dried powder.

1-2. Preparation of ethanol extract of grape seed

1 kg of dried and crushed grape seed was mixed with 10 folds volume of 100% ethanol and extracted with stirring for 12 hours at R. T. The extract was filtered and concentrated in vacuo to be 1/10 volume of extract. 50g of concentrates was mixed with equal amount of hexane and fractionated with repeated extraction to remove a hexane soluble layer. A remaining lower layer was concentrated and dried with lyopholization to obtain 30g of brown dried powder. The obtained extract was dissolved in water of 10mℓ, which was used as an undiluted solution (100mg extract/mℓ water) in following experiments.

Experimental Example 1. Inspection of cell protecting effect in low oxygen environment of grape seed extract- measurement of lactate dehydrogenase (*in vitro*)

PC12 cell was treated with $COCl_2$ to induce low oxygen environment to cause the injury of neuronal cell. To determine whether the injury of neuronal cell occur or not, the LDH concentration released from extra-cellular medium in culture cell was determined. The cell medium was collected at 20 to 24 hours after the treatment, i. e. , at the time of completing the release of enzyme, and the concentration of released enzyme was determined using by microplate reader.

The PC 12 cell was treated with various concentrations of grape seed extract, i. e., 0,10, 50,100, 500 and 1000 μg/ml, before and after the inducement of low oxygen en- vironment and cultivated for 20 to 24 hours at 37 °C to obtain cell culture medium. The concentration of LDH was determined using by Beckman DU-640 absorption spectrophotometer according to enzyme dynamic method using by Zhong Sheng Biotech standard reagent.

The result was shown in Fig. 1. The test groups treated with grape seed extract before and after the inducement of low oxygen environment reduced the release of LDH in dose dependant manner compared with those of control group treated with only solvent. Accordingly, it is confirmed that the grape seed extract reduced apoptosis induced by low oxygen environment significantly.

Experimental Example 2. Inspection of neuronal cell protecting effect of grape seed extract- animal experiment (*in vivo*)

The inhibiting effect of neuronal cell death in the grape seed extract was confirmed by the animal experiment using gerbils.

A. The Breeding of Experimental animal and administration method

60 numbers of male and female Mongolian gerbil (Meriones unguiculatus) weighing 65 to 75g were used as experimental animals.

The animals providing with free access to water and feed were acclimated with following breeding condition maintaining the temperature of 23 ± 2°C and the relative

humidity of 55 ± 10 °C under the regularly controlled light/dark condition, i. e. , light from am 7: 00 to pm 7: 00.

B. Experimental Procedure

0.5 mℓ of the grape seed extract was orally administrated into experimental animals 30 minutes before and after the inducement of ischemia as a test group and the non-treated group was used as control group. The experimental animals were orally anesthetized with 3% isoflurane gas (Baxtor Co., USA) in the gas mixture consisting of 70% nitrogen gas and 30% oxygen gas) and maintained with 2.5% isoflurane gas during the experiment. The disinfected skin at the center of mouse neck was excised and right-handed CCA (common carotid artery) and ECA (external carotid artery) were isolated from neighboring tissue and nerves with care. CCA and ECA were ligated for 5 minutes with aneurysm clip (Staelting, USA) to induce ischemia and the clips were removed to provide with repurfusion. Similar surgery to test group was performed as a control group. The complete occlusion of CCA in test groups was confirmed by observing the blood circulation of central artery of retina with ophthalmoscope. The body temperature of animals during the inducement of ischemia was determined by inserting thermometer into rectum and was maintained at 37 ± 0.3 °C using by automatically controlled heat pad according to the experimental temperature.

4 days after the ischemia inducement, each 40mg/kg amount of thiopental sodium (Yuhan Co., Korea) was intraperitoneally administrated to normal group, control group and test group respectively to anesthetize and physiological saline solution containing 1000 IU heparin per 1000mℓ solution maintaining the temperature of 4°C was injected into left ventricle to perfusive washing. Perfusion fixation was performed using 4% paraformaldehyde containing 0. 1M phosphate buffer (pH 7.4) and the brain of animal was delivered and fixed with fixation solution for 4 to 6 hours. The fixed brain was dipped in 30% sucrose solution in 0.1 M phosphate buffer. The brain was sliced into 30μm width of tissue slices using by sliding microtome (Reichert-Jung Co. Germany) and the slices were stored at 6 well plate containing storing solution at 4 °C before use.

The tissue wherein hippocampus formation was well developed was selected among the all slices and washed with 0. 01M PBS three times for 10 minutes to remove remaining storing solution. The slice was transferred to the gelatin coated slide and dried at 37°C sufficiently, dipped in distilled water for a while and stained with 2%

cresyl violet acetate (Sigma Co. USA). The tissue was washed with running tap water to remove remaining staining reagent, dipped in distilled water for a while, treated with 50%, 70%, 80%, 90%, 95% and 100% solution, dehydrated and remaining staining agent was removed by washing. After confirming the detection of Nssle body in the tissue, the tissue was dipped in xylen reagent (Junsei Co. Japan) to be transparent and sealed with Canadian Balsam (Kanto Co. Japan).

The CA1 region of each tissue was photographed by Axioplan microscope equipped with digital camera (Carl Zeiss Co. Germany) magnified with 1000x. The violet stained region was selected using by image analyzer (Optimas 6.5, USA) program and the number of neuronal cells was counted. To verify the significance for each group, the number of live neuronal cell was divided into that of normal cell and the result was expressed with percentage (%). One way ANOVA test was performed for statistical analysis and most general region selected from each group was photographed by Axioplan microscope equipped with digital camera (Carl Zeiss Co. Germany).

C. Experimental Result

Photographs of dyed hippocampus tissue of each experimental group were shown in Figs. 2, 3, and 4.

Fig. 2 shows photographs of dyed hippocampus tissue of a normal group (A and C) and a control group (B and D) in ischemia-reperfusion treatment of experimental animals. C and D are photographs magnified with 400× of the CA1 region of A and B.

As can be seen in Fig. 2, neuronal cell was observed in normal group while not detected in control group since all the neuronal cells were died from ischemia.

Figs. 3 and 4 show results in the experimental group administered with the grape seed extract. In Fig. 3, A and B are the photographs treated with grape seed extract 30 minutes before ischemia inducement whereas C and D are the photographs treated with grape seed extract 30 minutes after ischemia inducement. A and B are photographs of male while C and D are of female. As can be seen in Fig. 3, CA 1 region of tissues was stained with dark color, which means that all the test groups treated with grape seed extract show potent inhibiting activity of apoptosis. The photographs of Fig. 3 was photographed with 25×. The CA1 region of Fig. 3 was magnified with 400× to show Fig. 4. Fig. 4 shows that the cell shapes of test group and negative control

groups (A and C of Fig. 2) are similar to each other therefore, it is confirmed that the grape seed extract has potent inhibiting activity of neuronal cell death.

Fig. 5 shows the determined result of the experiment confirming the effect of grape seed on the apoptosis of neuronal cell after ishemia-reperfusion treatment.

The determined number of living neuronal cells for each group, i. e. , normal group Nrmal), control group (Control), test group treated with grape seed extract before ischemia-reperfusion treatment (pre-male and pre-female), test group treated with grape seed extract after ischemia-reperfusion treatment (post-male and post-female) was divided into that of negative control group and the calculated value was expressed as percentage. Asterisk (*) denotes the effective group with 99% of statistical significance level.

As can be shown in Fig. 5, the survival ratio of control group showed higher rate (about 11.6%) than that of normal group however, the survival ratio of test group showed highest rate among the groups. The survival ratio of neuronal cell in grape seed treated group to male and female animals showed 59.6 and 57.4 % in treatment group before the inducement of brain ischemia, whereas 51.9 and 71. 9% in treatment group after the inducement of brain ischemia, respectively.

Considering the fact that the survival rate of neuronal cell treated with positive control group (Ebselen) ranges from 50 to 60%, it proved the excellent protecting effect of neuronal cells of the grape seed extract in spite of a natural substance.

Accordingly, it is confirmed that the grape seed extract of the present invention can be useful in treating and preventing degenerative brain disease such as ischemia, stroke and so on as a medicine, health care food in spite of natural resource.

Experimental Example 3. Toxicity test

1. Oral administration

The acute toxicity tests on ICR mice and Sprague-Dawley rats were performed using the extract. Four group consisting of 10 mice or rats was administrated orally with 100, 250, 500 and 1000mg/kg of test sample or solvents respectively and observed for 2 weeks.

2. Non-oral administration

The acute toxicity tests on ICR mice (weight 25 ± 5g) and Sprague-Dawley rats were performed using the extract. Four group consisting of 10 mice or rats was administrated intraperitoneally with 25, 50, 100 and 200mg/kg of test sample or solvents, respectively and observed for 24 hours.

As experimental results, there was no toxicity in the grape seed extract of the present invention.

Hereinafter, the formulating methods and kinds of excipients will be described, but the present invention is not limited to them. The representative preparation examples were described as follows.

Preparation example. Composition for preventing ischemia neuronal cell injury

A. Preparation of injection

100mg of the grape seed extract of Example 1, 3.0g of sodium metabisulfite, 0.8mg of methylparaben and 0.1mg of propylparaben were mixed in distilled water for injection to obtain 2mℓ of injection. The injection was sterilized.

B. Preparation of tablet

200mg of the grape seed extract of Example 1, 100mg of lactose, 100mg of corn starch and 600mg of Magnesium Stearate were mixed and entableted to obtain a tablet.

C. Preparation of capsule

100mg of the grape seed extract of Example 1, 50mg of lactose, 50mg of corn starch, 600mg of Magnesium Stearate were mixed and charged in a gelatin capsule to obtain a capsule.

D. Preparation of liquid

100mg of the grape seed extract of Example 1, 20g of sugar, 20g of isomerized sugar and a proper dose of lemon perfume were mixed in distilled water to be the total

amount of 100mℓ. The resulting solution was charged in a brown bottle and sterilized to obtain a liquid.

E. Preparation of health food

1000mg of the grape seed extract of Example 1, vitamin mixture optimum amount (Vitamin A acetate 70μg, Vitamin E 1.0mg, Vitamin B1. 0.13mg, Vitamin B2 0.15mg, Vitamin B6 0.5mg, Vitamin B12 0.2μg, Vitamin C 10mg, Biotin 10μg, Amide nicotinic acid 1.7mg, Folic acid 50μg, Calcium pantothenic acid 0.5mg), and Mineral mixture optimum amount (Ferrous sulfate 1.75mg, Zinc oxide 0.82mg, Magnesium carbonate 25.3mg, Monopotassium phosphate 15mg, Dicalcium phosphate 55mg, Potassium citrate 90mg, Calcium carbonate 100mg, Magnesium chloride 24.8mg) were mixed. Other food addtives were added in the resulting mixture to obtain health food.

The above-mentioned vitamin and mineral mixture may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention.

F. Preparation of health beverage

100mg of the grape seed extract of Example 1, 1000mg of Citric acid, 100g of Oligosaccharide, 2g of Apricot concentration, 1g of Taurine were mixed in distilled water to be the total amount of 900mℓ. Health beverage preparation was prepared by dissolving active component, mixing, stirring at 85 °C for about 1 hour, filtered and then filling all the components in 2 liter ample and sterilizing by conventional health beverage preparation method.

The invention being thus described, it will be obvious that the same may be varied depending on local and racial characteristics including demand class, demand country and usage. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

Effect of the Invention

As described in the present invention, the grape seed extract shows potent inhibiting activity of neuronal cell apoptosis occurred in brain ischemia as well as no

toxicity, therefore, it can be used as the therapeutics or health food for treating and preventing neuro-degenerative brain diseases.

(57) What is Claimed is:

1. A grape seed extract having neuronal cell protecting activity obtained by a method that comprises:

(a) adding water of about 1 to 20-fold volume of water to the weight (kg) of a dried and crushed grape seed, stirring the water at the temperature ranging from 20 to 50 °C for about 1 hour to three days, and mixing with strong base like sodium hydroxide and potassium hydroxide adjusted to the pH ranging from 8 to 11 to stir the solution for about 1 to 24 hours;

(b) adding strong acid such as hydrochloric acid in the extract solution to adjust to the pH ranging from 2 to 4, and then centrifuging the solution to obtain a precipitated layer;

(c) adding about 3 to 7 fold weight of lower alcohol such as methanol or ethanol in the precipitant, and decompressing and concentrating the centrifuged solution to obtain its concentrate; and

(d) adding an organic solvent such as hexane in the concentrate with the same amount, and removing hexane which is a supernatant layer to purify and lyophilize the residual solution.

2. A composition comprising the grape seed extract as an active ingredient for the treatment and prevention of degenerative brain disease.

3. The composition according to claim 2, wherein said extract is selected from the group consisting of water, lower alcohol of C_1-C_4 and the mixture thereof.

4. The composition according to claim 3, wherein said extract is prepared by the process as set forth in claim 1.

5. The composition according to any of claim 2, wherein said degenerative brain disease comprises stroke, cerebral concussion, Parkinson's disease, Huntington's disease, Pick disease and Creutzfeld-Jakob disease.

6. A health food comprising the grape seed extract having neuronal cell protecting activity together with a sitologically acceptable food subsidiary additive.